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Metabarcoding of Bacterial Pathogens in a Rodent Pest: Which Organ?

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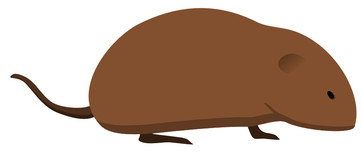
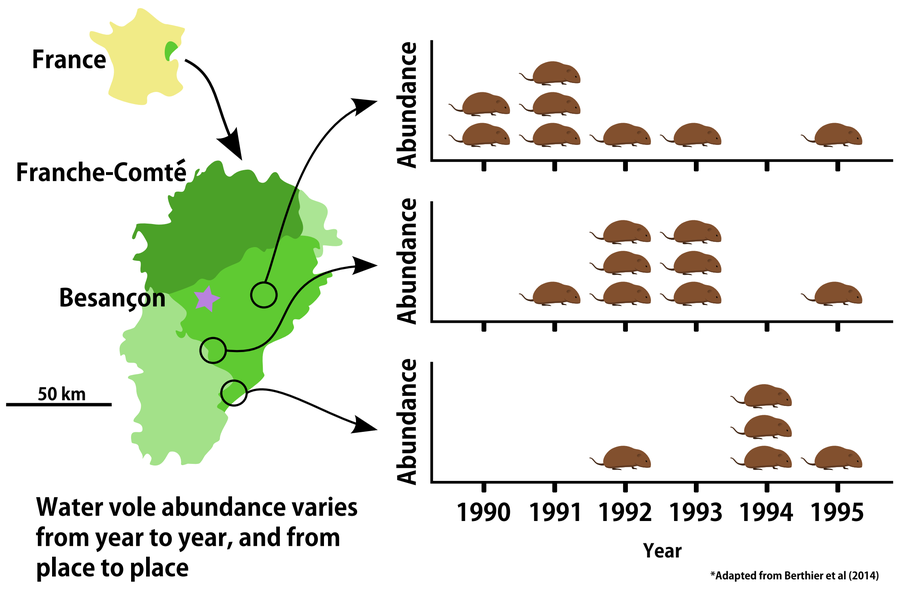
The water vole, *Arvicola terrestris*



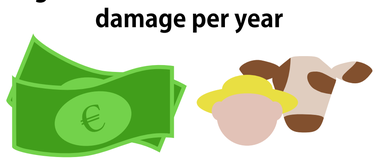
Water voles (*Arvicola terrestris*) are pests in Franche-Comté



Water vole abundance varies from year to year, and from place to place

*Adapted from Berthier et al (2014)

When they are abundant, they can cost a single farmer thousands of euros in damage per year



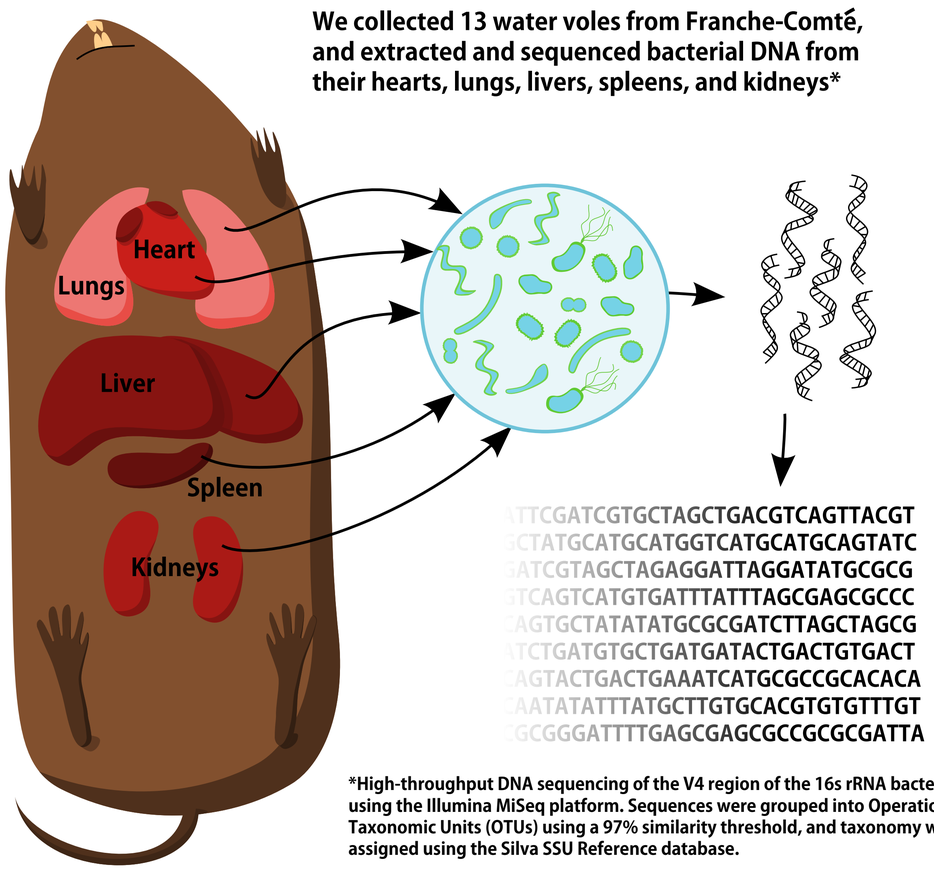
Water voles can carry tapeworms that are dangerous to humans

We know little about the bacteria hosted by this rodent; bacteria may drive changes in vole abundance, and may also pose a risk to human health

With which organ should we look for bacteria?

We can use DNA sequencing technology to search for bacteria, but we need to know which organs to look in.

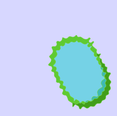
We collected 13 water voles from Franche-Comté, and extracted and sequenced bacterial DNA from their hearts, lungs, livers, spleens, and kidneys*



*High-throughput DNA sequencing of the V4 region of the 16s rRNA bacterial gene using the Illumina MiSeq platform. Sequences were grouped into Operation Taxonomic Units (OTUs) using a 97% similarity threshold, and taxonomy was assigned using the Silva SSU Reference database.

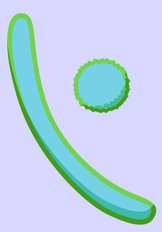
We found 25 potentially pathogenic bacteria in our voles

These are bacteria that we know can cause disease in animals, and may be causing disease in our voles. The 5 most prevalent bacteria we found:

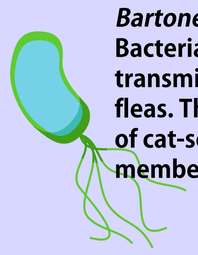


Acinetobacter
Common soil bacteria that can cause infections in already-sick animals

Treponema
The causative agents of yaws and human and rabbit syphilis are members of this genus



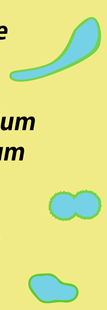
Mycoplasma
A diverse group, bacteria in this genus can infect mammals, birds, reptiles, fish, insects, and plants



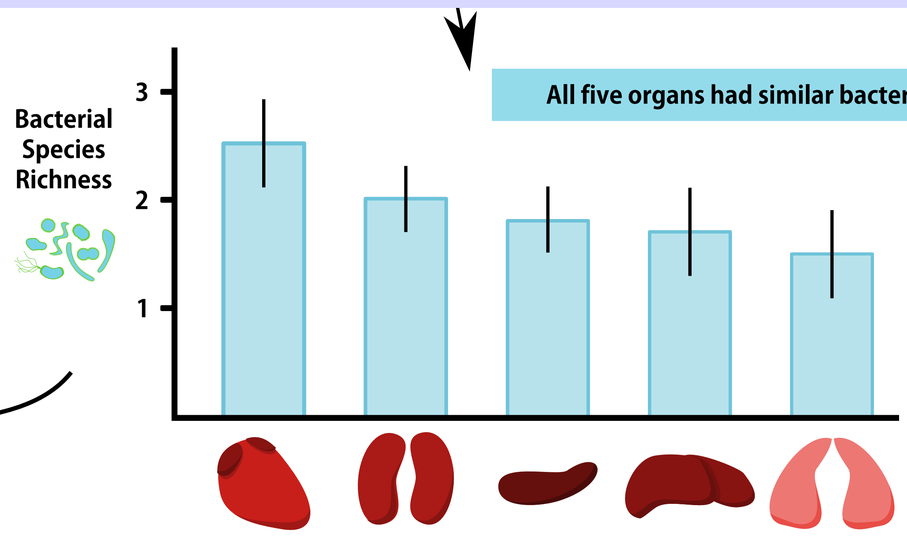
Bartonella
Bacterial parasites that are transmitted by ticks and fleas. The causative agent of cat-scratch disease is a member of this genus

Other bacteria we found:

Avibacterium
Pasteurellaceae
Helicobacter
Aerococcus
Chryseobacterium
Cornyebacterium
Leptospira
Peptococcus
Sphingomonas
Streptococcus
Ureaplasma

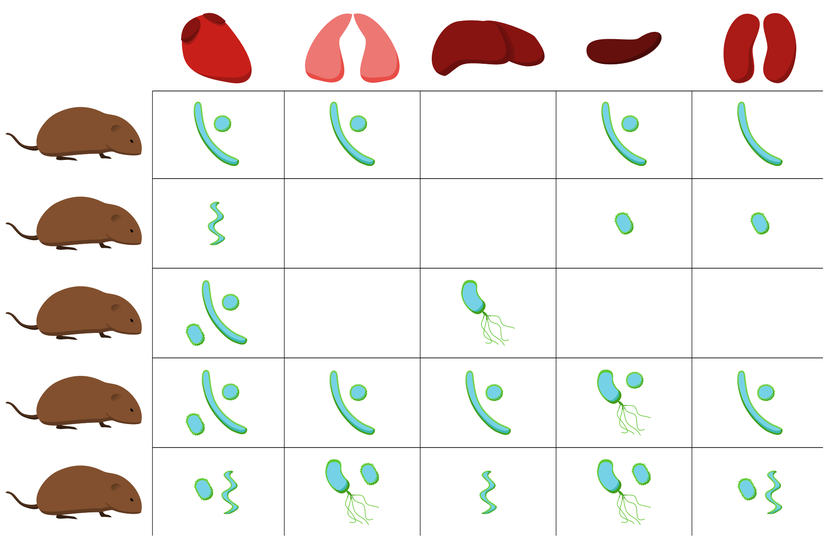


All five organs had similar bacterial species richness *

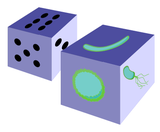


*Mean bacterial species richness ranged from 1.5-2.5 species/organ, and did not differ significantly between organs (generalized linear mixed-effects model for average OTU richness within organs, with animal as a random effect. The model was fit using maximum likelihood, a Poisson distribution, and log-link function). Error bars are standard error.

We found different bacteria in different organs



But...

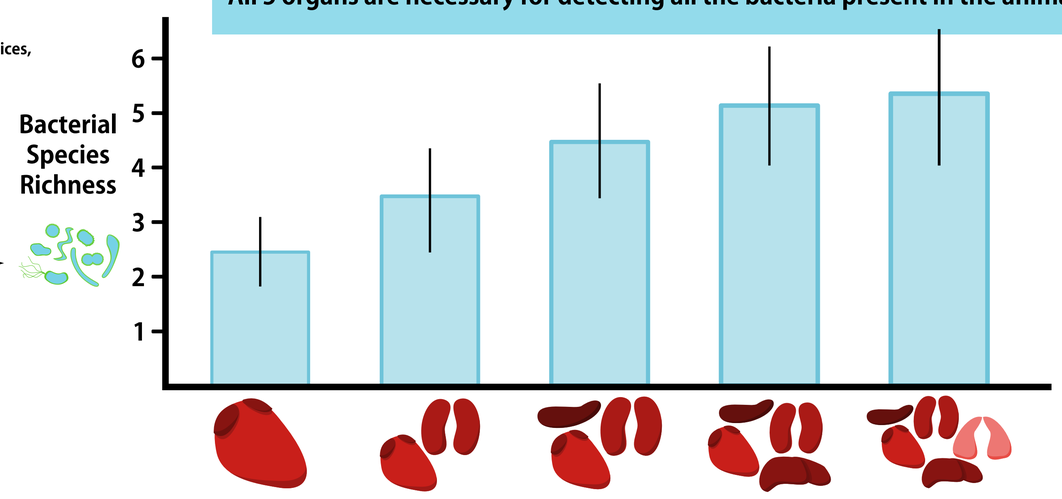


These patterns of different bacteria in different organs appear random*

*PERMANOVA with Jaccard dissimilarity indices, pseudo-$F_{4,51}$ = 1.34, p=0.120

And...

All 5 organs are necessary for detecting all the bacteria present in the animal*



*Bootstrapped mean bacterial richness as organs are included in the richness calculation for each animal. Error bars are bootstrapped 95% confidence intervals (999 iterations for means and confidence intervals). Increasing mean richness with increasing nmber of organs indicates that organs differ in the bacterial they host, and that bacteria are not concentrated in one or two organs

If we pool all organs within an animal, we find an average of 5.4 bacterial species per animal*

*5.4 ± 0.7 (mean ± standard error), which is 2.9 ± 0.9 species (bootstrapped mean and 95% confidence interval) greater than the mean bacterial richness of the heart, the organ with the highest bacterial richness.

Conclusion: We should use all five organs when we are looking for bacteria in water voles

Different organs host different bacteria, and bacteria are not concentrated in a single organ

In addition, all 5 organs are necessary to detect host-population-level differences in bacterial assemblages. The animals sampled here are from two different populations; we found that organ bacterial richness does not vary with location (generalized linear mixed-effects model for average OTU richness within organs, with animal and location as random effects, fit using maximum likelihood, a Poisson distribution, and log-link function), but differences in pooled, liver, and lung bacterial assemblages map to host populations (ordination and PERMANOVA analysis of Jaccard dissimilarities). Livers and lungs are prone to having no detectable bacteria, making them poor candidates for studying host-population level bacterial assemblages, thus pooling organs within animals is the "best" choice.

Berthier, K., Piry, S., Cosson, J.-F., Giraudoux, P., Foltête, J.-C., Defaut, R., Truchetet, D., Lambin, X. (2014) Dispersal, landscape, and travelling waves in cyclic vole poulations. Ecology Letters, 17:53-64